

**Bonneville Power Administration
Fish and Wildlife Program FY99 Proposal**

Section 1. General administrative information

**Performance/Stock Productivity Impacts of
Hatchery Supplementation**

Bonneville project number, if an ongoing project 9005200

Business name of agency, institution or organization requesting funding
Biological Resources Division, U.S. Geological Survey (formerly National Biological Survey)

Business acronym (if appropriate) BRD

Proposal contact person or principal investigator:

Name	<u>Reg Reisenbichler</u>
Mailing Address	<u>6505 NE 65th St.</u>
City, ST Zip	<u>Seattle, WA 98115</u>
Phone	<u>206-526-6282 x334</u>
Fax	<u>206-526-6654</u>
Email address	<u>Reg_Reisenbichler@usgs.gov</u>

Subcontractors.

Organization	Mailing Address	City, ST Zip	Contact Name
University of Idaho	Idaho Cooperative Fish and Wildlife Research Unit, College of Forestry	Moscow, ID 83843	Dr. Ted Bjornn
University of Washington	Washington Cooperative Fish and Wildlife Research Unit, School of Fisheries, Box 357980	Seattle, WA 98195	Dr. Chris Grue
U.S. Fish and Wildlife Service	Fisheries and Federal Aid, 911	Portland, OR 97232-4181	Carolyn Standfill

	NE 11th Ave.,		
Washington Dept. of Fish and Wildlife	Natural Resources Bldg., P.O. Box 43151	Olympia, WA 98504-3151	Dr. Jim Shaklee

NPPC Program Measure Number(s) which this project addresses.

7.3B.2

NMFS Biological Opinion Number(s) which this project addresses.

Other planning document references.

Subbasin

N/A. Although the study is conducted in the Clearwater, Deschutes, and Little White Salmon river systems, the results apply to or affect all sub-basins throughout the Columbia River system and along the West Coast where supplementation is conducted or proposed.

Short description.

Provide increased understanding of the reputed failure of supplementation with steelhead in Idaho's Clearwater River, and an improved basis for planning and modifying supplementation programs and evaluations of these programs by measuring genetic effects from artificial propagation.

Section 2. Key words

Mark	Programmatic Categories	Mark	Activities	Mark	Project Types
X	Anadromous fish		Construction		Watershed
	Resident fish		O & M		Biodiversity/genetics
	Wildlife		Production		Population dynamics
	Oceans/estuaries	X	Research		Ecosystems
	Climate		Monitoring/eval.		Flow/survival
	Other		Resource mgmt		Fish disease
			Planning/admin.	X	Supplementation
			Enforcement		Wildlife habitat en-
			Acquisitions		hancement/restoration

Other keywords.

Genetics, hatchery-wild interactions, domestication, fitness for natural rearing

Section 3. Relationships to other Bonneville projects

Project #	Project title/description	Nature of relationship
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Section 4. Objectives, tasks and schedules

Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	Compare the growth and survival of genetically marked offspring from wild, Clearwater River, steelhead (W) and from Dworshak National Fish Hatchery (Dworshak NFH) steelhead (H) rearing together in two natural streams in the Clearwater River system, Idaho. Comparisons of HxH and WxW fish are to be completed for each of four year-classes. HxW fish will be included for at least one year-class to test for maternal effects. Growth (in length and weight) and survival will be evaluated at the end of each growing season, and at the time of downstream migration.	1a	Sample hatchery fish at Dworshak NFH, and capture wild adults at Selway Falls. Determine the dipeptidase (PEPA) genotypes of adults; retain only wild fish homozygous for the common allele (up to 30 females and 20 males), only hatchery fish homozygous for the alternate allele. Combine gametes to create genetically marked groups of HxH, HxW, and WxW fish at the PEPA locus. Adjust the incubation temperatures so that the experimental groups reach the button-up stage simultaneously. Release these experimental groups of fish as swim-up fry in Twenty-mile and Silver Creeks.
		1b	Sample juveniles residing in the streams at the end of each growing season, and trap juveniles migrating downstream. Determine relative survival and size of the offspring of HxH, HxW, and WxW fish. Estimate the standing stock of experimental fish in the streams and the number of outmigrants, and account for differential outmigration.
2	Compare the growth and survival of genetically marked	2a	Capture hatchery and wild adults, combine their gametes to create

	<p>offspring of wild spring chinook salmon from Warm Springs River (W) and of Warm Springs Hatchery fish (H) in two natural streams near the Deschutes River system, Oregon. Comparisons of HxH and WxW fish will be completed for each of four year-classes. HxW fish will be included in at least one year-class. Growth (in length and weight) and survival will be evaluated at the end of each growing season, and at the time of downstream migration.</p>		<p>genetically marked groups of HxH, HxW, and WxW fish at the superoxide dismutase (s-SOD) locus, and release these experimental groups of fish as eyed embryos or swim-up fry in the Little White Salmon River and in Buck Creek, tributary to the Big White Salmon River.</p>
		2b	<p>Sample juveniles residing in the study streams at the end of each growing season, and trap juveniles migrating downstream. Determine relative survival and size of the offspring of hatchery and wild fish.</p>
3	<p>Compare the growth and survival of genetically marked offspring from local wild fish (W) and from hatchery fish (H) in hatchery ponds at Dworshak, Clearwater, or Warm Springs hatcheries. Comparisons of HxH and WxW fish will be completed for each of four year-classes. HxW fish will be included in at least one year-class. The two groups of fish will be reared together (i.e., in the same ponds). Growth (in length and weight) and relative survival will be evaluated immediately before the juvenile fish are released from the hatchery as smolts. Steelhead will be reared at Dworshak or Clearwater hatcheries; spring chinook salmon will be reared</p>	3a	<p>Rear genetically marked HxH, HxW, and WxW groups of fish, spawned in Tasks 1a or 2a, together in hatchery troughs or ponds using standard hatchery rearing practices and release the fish at the standard time of release.</p>

	at Warm Springs National Fish Hatchery.		
		3b	Compare the relative survival and size of experimental fish shortly before the standard release date.
		3c	Mark the experimental fish with an external mark so that they can be recognized when they return as adults. Determine relative return rates and sizes of these fish when they return as adults. Approximately 100 juvenile spring chinook salmon will be transported to BRD's Marrowstone Field Station where their growth and survival in sea water will be monitored for three to six months.
4	Compare the reproductive success (the relative survival of offspring) of genetically marked adult offspring from wild steelhead (WxW) and from hatchery steelhead (HxH) spawning in Silver Creek or Crooked River, tributaries of South Fork Clearwater River. The comparison is to be made for each of four year-classes.	4a	Develop length-fecundity and weight-fecundity relations for hatchery fish and for wild fish spawned for Objectives 1 and 3. Determine whether the size of eggs differs between hatchery fish and wild fish.
		4b	Capture adult fish returning to Dworshak NFH and to the Selway River as in Task 1a, or adult experimental fish returning to Crooked River. Release radio-tagged adults with the appropriate (homozygous) PEPA genotypes into Silver Creek above a weir to prevent them from leaving the stream before spawning. Estimate the number of eggs in hatchery females and in wild females released above the barriers. Using radio telemetry, ascertain whether any adults leave the study section before spawning, and whether there are differences in spawning

			location and habitat between hatchery and wild fish.
		4c	Sample the juveniles, offspring of the hatchery and wild adults released into the study sections, residing in each stream at the end of each growing season to determine relative reproductive success and size of the offspring of hatchery fish and wild fish. Trap the juveniles that migrate downstream, and test for differences in migration between groups.
5	Test for selection on the genetic marks by comparing the growth and survival of juvenile fish with the different genotypes rearing together in natural streams and in hatcheries. The test fish will be the offspring of hatchery fish. The tests will be repeated for up to four year-classes, depending on preliminary results from the first two year-classes.	5a	At the respective hatcheries, mate adults heterozygous at the locus used for genetic marks (sSOD-1 or PEPA). Release at least 20,000 of the resulting fish (from each year-class) in a study stream, and rear at least 60,000 in the hatchery.
		5b	Take a sample of juvenile fish from the study stream at the end of each growing season, and determine the mean size and relative survival of fish with the different genotypes.
		5c	Compare the relative survival and size of experimental fish with the different genotypes immediately before the standard release date at the hatchery.
		5d	Determine relative return rates and sizes of fish with the different genetic marks when they return to the hatchery as adults.
6	Test for an effect of incubation	6a	Spawn adult steelhead at

	temperature by comparing the growth and survival of juvenile steelhead from matings early in the season, incubated at 7EC and from matings made later, incubated at 12EC. Crosses will be timed to result in simultaneous emergence for the two groups.		Dworshak NFH on the appropriate dates and incubate at the respective temperatures to achieve simultaneous button-up. Mark the otoliths of the juveniles thermally. Release approximately one-half of the resulting fish (from early and from late matings) as unfed fry in the North Fork Palouse River and rear the remainder in the hatchery.
		6b	Take a sample of juvenile fish from the study stream at the end of each growing season, and determine the mean size and relative abundance of fish from the two treatments (early, 7EC vs. late, 12EC).
		6c	Compare the relative survival and size of early and late experimental fish immediately before the standard release date at the hatchery.
		6d	Determine relative return rates and sizes of fish with the different genetic marks when they return to the hatchery as adults.
7	Test for an effect of cryopreservation by comparing the growth and mortality, and the response to various stressors of juvenile fish from Afresh \equiv and cryopreserved milt. Juvenile fish will be reared at the Western Fisheries Research Center (WFRC) and subsets will be challenged with (1) a fish pathogen, (2) restricted ration, (3) an acute handling stress, (4) a 48-hr density stress, and (5) a saltwater challenge.	7a	Take milt from at least 12 hatchery males. Divide the milt into two equal portions; cryopreserve one portion and hold the other portion in plastic bags filled with oxygen and held on ice. Take the eggs from the same number of female hatchery fish. Divide the eggs from each female into two equal portions, and fertilize one portion with the cryopreserved milt from one male, and the other portion with Afresh \equiv milt from the same male. Use a different male with each female.
		7b	Split each treatment (fresh or

			<p>cryopreserved milt) group for each mating, and incubate separately at WFRC until button-up. Divide each family and assign to rearing vessels so that each family is represented equally in each vessel. Two sets of tanks will contain fish from both treatments from all families, distinctively (marked by treatment) with mirror fin clips. One set of these mixed tanks will receive high ration levels (1.5 times the level recommended by the feed manufacturer). The other set will be fed at one-half of the recommended level. The remaining fish will be reared separately by treatment (with all families equally represented in each tank), and fed at the high level.</p>
		7c	<p>Monitor the growth and survival, by treatment, of the fish in the mixed tanks, and test for differences between treatments. During December-February, as the fish approach age-1, divide the fish from the unmixed tanks into replicate vessels, and evaluate (1) time to death and percent mortality for fish subjected to <u>Vibrio anguillarum</u>, (2) blood cortisol levels of fish (2a) held in a dip net out of the water for 60 seconds (acute handling stress) and (2b) fish held at high density in buckets with approximately 5 cm of water for various periods, up to 48 hours, and (3) blood sodium levels of fish held in sea water for 24 hr.</p>

Objective schedules and costs

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Objective #	Start Date mm/yyyy	End Date mm/yyyy	Cost %
1	06/1991	Dec/1999	25
2	06/1991	Dec/2001	15
3	06/1991	Dec/2002	20
4	01/1992	Dec/2002	12
5	06/1991	Jul/2002	20
6	01/1994	Jun/2000	5
7	01/1996	Dec/1998	3

Schedule constraints. Identify any constraints that may cause schedule changes. Describe major milestones if necessary.

Arbitrary budget reductions, such as the arbitrary reduction of our budget for 1998 by \$41,000, will postpone or preclude completion of objectives because we will have inadequate manpower to accomplish the field and lab work and report preparation.

Completion date. Enter the last year that the project is expected to require funding.
2002

Section 5. Budget

FY99 budget by line item

Item	Note	FY99
Personnel		157,973
Fringe benefits		45,022
Supplies, materials, non-expendable property		48,756
Operations & maintenance		11,832
Capital acquisitions or improvements (e.g. land, buildings, major equip.)		0
PIT tags	# of tags:	0
Travel		18,312
Indirect costs		111,615
Subcontracts		102,983
Other		0
TOTAL		496,494

Outyear costs

Outyear costs	FY2000	FY01	FY02	FY03
Total budget	430,000	335,000	315,000	0
O&M as % of total	2.5	2.5	2	0

Section 6. Abstract

The methods involve crossing adult fish to produce genetically marked progeny of hatchery x hatchery, hatchery x wild, and wild x wild parentage. The progeny are incubated in identical conditions in the hatchery until button-up when the groups are mixed together. Mixed populations are divided; some fish are released into natural streams, others are reared in the hatchery. The progeny are sampled before or during their downstream migration or as returning adults to determine relative growth and survival of the treatment groups. The relative performances will be used to infer effects on stock productivity from using progeny of wild fish in the hatchery and hatchery fish in streams, and to design evaluations. The study will be completed in December 2002.

Section 7. Project description

a. Technical and/or scientific background.

Supplementing spring chinook salmon and steelhead is expected to contribute much toward doubling the runs in the Columbia River system. However, supplementation programs pose a risk to the supplemented populations of wild fish because these programs may reduce the productivity of the wild population and its ability to adapt to altered or changing environmental conditions (e.g., Hindar et al. 1991). Such reductions might result from genetic changes in the wild population which occur when hatchery fish interbreed with wild fish.

This study provides data for planning and evaluating supplementation which is a conspicuous element in the various plans of the Northwest Power Planning Council and the Columbia River Intertribal Fisheries Commission to restore salmon and steelhead in the Columbia River system. Uncertainties and risks associated with supplementation have featured prominently in NMFS's status reviews for endangered species and in reviews of the Council's restoration program by the National Research Council (National Research Council 1996) and the Independent Science Group (1996).

Data indicate that hatchery fish or their progeny do not survive or reproduce in natural streams as well as do wild fish because of genetic differences (Reisenbichler and McIntyre 1977; Chilcote et al., 1986; Nickelson et al., 1986; Leider et al. 1990). Nevertheless, managers are concerned that these data derive from a very limited number of studies -- studies of only steelhead or coho salmon; each study with substantial limitations -- and that results from these studies may not be generally applicable. Consequently, managers often are unsure of the necessity for replacing or modifying existing hatchery stocks (by developing a new hatchery population from wild broodstock or by including substantial numbers of wild fish in the existing brood stock, respectively) that have performed well in the hatchery.

The studies cited above suggest that the fitness of the wild population in natural waters will decline when hatchery fish interbreed with wild fish. The greater the genetic

difference between hatchery and wild fish, the greater the expected effect on the wild population, hence managers wish to minimize the genetic differences between hatchery and wild fish. Some managers have initiated or planned supplementation programs where only the offspring of wild fish are reared in the hatchery, expecting that the genetic effect from only one (consecutive) generation in the hatchery environment is negligible. This assertion requires further consideration, testing and quantification, particularly in view of Reisenbichler and McIntyre's work (1977) which showed significant genetic differences in growth and survival (both in streams and the hatchery) between wild steelhead and hatchery steelhead derived from the wild stock only two generations before the comparison. One should expect that most of that genetic change occurred in the first generation!

Certain practices can help to reduce the genetic difference between hatchery and wild fish. Such practices are thought to include (1) developing the hatchery stock from local wild fish, (2) including wild fish in the brood stock each year, (3) avoiding selective breeding in the hatchery for nonadaptive traits (such as large body size or early time of spawning), and (4) modifying the hatchery environment so that natural selection effects little change of the original wild genome. Although such practices have been recommended since 1977 or before, their efficacies remain to be demonstrated. This study constitutes a partial evaluation of these recommended practices, and also provides information for inferring the amount of genetic change expected in a stock of fish after a single generation in the hatchery.

This study evaluates (i) the costs and benefits (relative growth, survival, and reproductive success) of alternative sources (hatchery vs. wild) for hatchery brood stocks in supplementation programs, (ii) whether a hatchery stock (Warm Springs National Fish Hatchery) initiated with local, wild fish and supplemented with wild fish in the brood stock each year has been effective at keeping hatchery fish genetically similar to the wild fish, and (iii) whether environmental conditions in the hatchery can be modified to reduce genetic differences between hatchery and wild fish while retaining some minimum level of economic efficiency. The first two elements involve testing for genetic differences between hatchery and wild populations to illustrate whether existing data are applicable to steelhead in general and whether similar genetic differences between hatchery and wild fish occur in spring chinook salmon. The resulting data should be extremely valuable in planning whether or not to supplement, how much to supplement, what fish to use as broodstock, whether modifications of the hatchery environment are worth considering, and designing evaluation programs for supplementation projects (Reisenbichler in prep.).

Bonneville Power Administration and others have funded projects which include evaluating specific supplementation programs; however, these evaluations are designed to test for increased production, not to measure genetic differences in growth and survival between hatchery and wild fish. The former studies are extremely important but require many years (more than 20 years; see below) before the gene pool (hence the combined

productivity for hatchery fish and naturally spawned fish) is expected to approach a steady-state. The latter studies (including this study) will be extremely useful both now and in the future for conceptualizing the fundamental (genetic) changes that occur with hatchery rearing and supplementation, and will thereby provide an improved basis for understanding supplementation, predicting its consequences, and designing rigorous evaluations. For example, preliminary modeling (Reisenbichler in prep.) suggests that the expected (combined hatchery and natural) production from a supplementation program for steelhead may be only one-half of that expected without accounting for genetic changes from natural selection in the hatchery program, even if only wild fish are used as brood fish in the hatchery. However, production from this program initially increases for several generations before declining through at least generation eight (30+ years for B-run steelhead and many spring chinook salmon populations) to near the final (low) equilibrium level. Knowledge of such a trajectory should help ensure well-designed evaluation programs of adequate duration.

b. Proposal objectives.

For simplicity the objectives will be presented separately for steelhead and spring chinook salmon. The hypotheses are given for both goals jointly, and follow the listing of objectives and tasks.

GOAL I: Compare the growth, survival, and reproductive success, in natural streams and hatcheries, of steelhead from established hatchery stocks and from wild stocks.

Objective I.1. Compare the growth and survival of genetically marked offspring from wild Selway River steelhead (W) and from Dworshak National Fish Hatchery (Dworshak NFH) steelhead (H) rearing in Twenty-mile and Silver Creeks (tributaries of the South Fork Clearwater River, Idaho). HxH and WxW fish will be compared for each of two year-classes; at least one year-class of HxW fish will be included. Growth (in length and weight) and survival will be evaluated at the end of each growing season, and at the time of downstream migration.

Objective I.2. Compare the growth and survival of genetically marked offspring from wild Selway River steelhead (W) and from Dworshak NFH steelhead (H) in hatchery ponds at Clearwater Hatchery. The experimental groups of fish will be reared together (i.e., in the same ponds). Growth (in length and weight) and relative survival will be evaluated immediately before the juvenile fish are released from the hatchery as smolts.

Objective I.3. Compare the reproductive success (the number of offspring produced) of genetically marked adult offspring from wild Selway River steelhead (WxW) and from Dworshak NFH steelhead (HxH) spawning in Silver Creek or Crooked River. The comparison will be made for each of at least two year-classes.

Objective I.4. Test for selection on the genetic marks (PEPA locus) by comparing the growth and survival of juvenile fish with the different genotypes rearing together in the Palouse River drainage and in Dworshak NFH. The test fish will be the offspring of hatchery fish. The tests will be repeated for at least two year-classes of fish.

Objective I.5. Test for an effect of incubation temperature by comparing the growth and

survival of juvenile hatchery fish from crosses made early in the season, incubated at .7EC; and juvenile hatchery fish from crosses made later in the season, incubated at .12EC. The specific temperature regimes will be scheduled to achieve the button-up stage of development simultaneously for early and late fish. (This objective was added because it was necessary to incubate HxH and WxW fish at different temperatures for the 1992 year-class to achieve simultaneous button-up. The effect of the manipulation is unknown, although presumed to be slight.)

Objective I.6. Test for an effect of cryopreservation by comparing the growth and mortality, and the response to various stressors of juvenile fish from Afresh \equiv and cryopreserved milt. Juvenile fish will be reared at the Western Fisheries Research Center (WFRC) and subsets will be challenged with (1) a fish pathogen, (2) restricted ration, (3) an acute handling stress, (4) a 48-hr density stress, and (5) a saltwater challenge.

GOAL II. Compare the growth and survival, in natural streams and hatcheries, of juvenile spring chinook salmon from Warm Springs National Fish Hatchery (Warm Springs NFH, a hatchery with almost continual, intentional gene flow from wild [Warm Springs River] salmon), Carson National Fish Hatchery (which has a "typical, well-established" hatchery population), and (the wild population of the) Warm Springs River.

Objective II.1. Compare the growth and survival of genetically marked offspring of hatchery fish from Warm Springs NFH (H) with those of wild fish from Warm Springs River (W) and hatchery fish from Carson National Hatchery (H') rearing in two study streams. HxH, H'xH', and WxW fish are to be compared for each of two year-classes. An additional year-class will include a HxW cross using wild males and hatchery females instead of the H'xH' cross to evaluate maternal effects. Growth (in length and weight) and survival will be evaluated at the end of the growing season, and at the time of downstream migration.

Objective II.2. Compare the growth and survival of genetically marked offspring from Warm Springs NFH fish (H) and wild Warm Springs River fish (W) rearing together in hatchery ponds at Warm Springs NFH. HxH, HxW, and WxW fish are to be compared for each of three year-classes. Growth (in length and weight) and relative survival will be evaluated shortly before the juvenile fish are released as smolts, and as returning adults.

Objective II.3. Test for selection on the genetic marks (at the s-SOD-1 locus) by comparing the growth and survival of juvenile fish with the different genotypes rearing together in the Little White Salmon River and in Warm Springs NFH. The test fish will be the offspring of hatchery fish. The tests are to be repeated for three year-classes of fish.

Objective II.4. Test for a maternal effect on the differences between experimental groups of fish. For the 1993 year-class, compare the growth and survival of thermally marked offspring from Warm Springs NFH fish (H'), Carson NFH fish (H), and the reciprocal crosses between these groups (Warm Springs NFH females x Carson NFH males vs. Carson NFH females x Warm Springs NFH males) rearing in the hatchery. (A test for maternal effects also will occur with the 1996 year-class, where a HxW cross, with only female hatchery fish and male wild fish, will

be included in Objectives II.1 and II.2.) HxH, HxH', H=xH (1993 year-class), and H'xH' fish will be reared together in the same ponds and streams. Growth (in length and weight) and relative survival will be evaluated shortly before the juvenile fish are released from the hatchery as smolts and as returning adults (and for the portion of the 1996 year-class rearing in the stream, at the end of the first summer and at the time of smolt outmigration.) The following tasks apply to the 1993 year-class; those for the 1996 year-class are included in tasks under Objectives II.1 and II.2.

Hypotheses:

Hypothesis 1. Survival in natural streams, from release to the end of each growing season and to downstream migration, does not differ among juveniles resulting from matings of HxH and WxW fish.

Hypothesis 2. Growth in natural streams, from release to the end of each growing season and to downstream migration, does not differ among juveniles resulting from matings of HxH and WxW fish.

Hypothesis 3. Survival in the hatchery, from fertilization to the standard time of release at each station and to returning adult, does not differ among fish resulting from matings of HxH and WxW adults.

Hypothesis 4. Growth in the hatchery, from fertilization to the standard time of release at each station and to returning adults, does not differ among fish resulting from matings of HxH and WxW adults.

Hypothesis 5. Reproductive success to swim-up fry is the same for offspring of HxH fish and WxW fish.

Hypothesis 6. Survival from swim-up fry to fingerling in late August and from swim-up fry to smolt is the same for offspring of naturally spawning HxH fish and WxW fish.

Hypothesis 7. Growth to late August is the same for offspring of naturally spawning HxH fish and WxW fish.

Hypothesis 8. Size of smolts and timing of downstream (smolt) migration in the spring is the same for offspring of naturally spawning HxH fish and WxW fish.

Hypothesis 9. Survival of juvenile fish from release to the end of the first summer (chinook salmon) or second summer (steelhead) in natural streams is independent of their genetic mark.

Hypothesis 10. Growth of juvenile fish to the end of the first summer (chinook salmon) or second summer (steelhead) in natural streams is independent of their genetic mark.

Hypothesis 11. Survival of juvenile fish in the hatchery and in a natural stream from fertilization to the time of release or migration as smolts is independent of their genetic mark.

Hypothesis 12. Growth of juvenile fish in the hatchery and in a natural stream to the time of release or migration as smolts is independent of their genetic mark.

Products: Final reports and publications describing the results of this study, and utilizing the results in conceptual (and perhaps quantitative) models to increase our understanding of the genetic issues of supplementation. These products should facilitate effective planning and evaluation of supplementation programs, and result in more effective supplementation.

c. Rationale and significance to Regional Programs.

The rationale and significance to Regional Programs was included in the response to 6a above. Please go to 6a above.

d. Project history (for continuing projects).

Major results achieved: Preliminary results for steelhead are consistent with those from previous studies, and illustrate adaptation of hatchery fish to the hatchery environment (domestication, which is beneficial for production hatchery programs) and loss of fitness for natural rearing (detrimental for supplementation programs). The weight of such evidence for steelhead should be compelling if our preliminary results stand when our experiments and analyses are complete. We have also documented behavioral differences between juvenile progeny of hatchery and wild steelhead which may suggest alterations of hatchery environments that can reduce the effect of domestication selection.

Preliminary results from spring chinook salmon suggest that despite the very different juvenile life history (habitat preferences; social behavior; length of residence in freshwater; ...) for chinook salmon and steelhead, hatchery rearing also reduces the fitness of spring chinook salmon for natural rearing. Indeed, use of wild fish to comprise 10-30% of the hatchery broodstock almost every year was insufficient to avoid loss of fitness for natural rearing. To our knowledge, these are the first data from tests of genetic differences in fitness between hatchery and wild chinook salmon. Without these data it was unknown whether hatchery rearing effects a loss of fitness for natural rearing, and how great that loss might be.

Technical papers and reports:

- Reisenbichler, R.R., and G.S. Brown. 1995. Is Genetic Change From Hatchery Rearing of Anadromous Fish Really a Problem? Pages 578-579 in Uses and Effects of Cultured Fishes in Aquatic Ecosystems. American Fisheries Society Symposium 15. Bethesda, MD.
- Brown, G.S., and R.R. Reisenbichler. 1996. Evidence for genetic change in behavior and survival of a hatchery population of steelhead, *Oncorhynchus mykiss*. Poster presented at the Ecological and Evolutionary Ethology of Fishes Conference, Albuquerque, New Mexico.
- Reisenbichler, R.R. 1996a. Effects of supplementation with hatchery fish on carrying capacity and productivity of naturally spawning populations of steelhead. Pages 81-92 in G.E. Johnson, D.A. Neitzel, and W.V. Mavros [eds.] Proceedings from a Workshop on Ecological Carrying Capacity of Salmonids in the Columbia Basin: Measure 7.1A of the Northwest Power Planning Council's 1994 Fish and Wildlife Program, Report 3 of 4. Bonneville Power Administration, Portland, OR.
- Reisenbichler, R.R. 1996b. The risks of hatchery supplementation. *The Osprey* 27: 1-4.
- Reisenbichler, R.R. 1997. Genetic factors contributing to declines of anadromous salmonids in the Pacific Northwest. Pages 223-244 in D.J. Stouder, P.A. Bisson, and R. J. Naiman [eds.] *Pacific Salmon and Their Ecosystems: Status and Future*

Options. M.G. Duke [assoc. ed]. Chapman & Hall, Inc., New York.
Reisenbichler, R.R. (in press). Questions and partial answers about supplementation--genetic differences between hatchery and wild fish. In Proceedings of the Columbia River Anadromous Salmonid Rehabilitation Symposium, Richland, WA (June 1995).

At least two, and probably five, manuscripts resulting directly from this work will be prepared and submitted for publication in primary fisheries journals.

Adaptive management implications: Data forthcoming from this study will be useful for conceptualizing risks from supplementation for both steelhead and spring chinook salmon as described in c. above. Also as described above, these data will facilitate better decisions on whether to supplement particular populations and if so, to better plan the supplementation and its evaluation.

Preliminary results from this study, in conjunction and consistent with data from three similar studies, have provided a basis for estimating the efficacy of supplementation programs for steelhead, and suggest that the actual benefit (adult production) from supplementation programs may be only a fraction (e.g., $<1/2$) of that expected without considering genetic changes from hatchery rearing. Where hatchery fish could be managed separately from wild fish--avoiding problems from mixed-stock fisheries, competition, predation, interbreeding, etc.--total production might be substantially higher by using the hatchery for a traditional production or harvest augmentation program, rather than for supplementation. Although preliminary, our results are sufficient to demand caution in universally using supplementation to increase salmon and steelhead production. Prudent planning and justification of supplementation must evaluate and include the expected genetic consequences on carrying capacity, production, and productivity of the naturally spawning population.

Years underway: 6.

Past costs: \$2,323,206 to 31 March 1998.

e. Methods.

Goals and objectives are repeated here for reference with their respective tasks.

Task I.1.1. Sample hatchery fish at Dworshak NFH, and capture wild adults (up to 30 females and 20 males) at Selway Falls. Determine the dipeptidase (PEPA) genotypes of adults; retain only wild fish homozygous for the common allele, only hatchery fish homozygous for the alternate allele. Combine gametes to create genetically marked groups of HxH, HxW, and WxW fish at the *PEPA* locus. Adjust the incubation temperatures so that the experimental groups reach the button-up stage simultaneously. Release these experimental groups of fish as swim-up fry in Twenty-mile and Silver Creeks.

Task I.1.2. Sample juveniles residing in the streams at the end of each growing season, and trap juveniles migrating downstream. Determine relative survival and size of the offspring of HxH, HxW, and WxW fish. Estimate the standing stock of experimental fish in the streams, and the number of outmigrants to allow accounting for differential outmigration.

Task I.2.1. Rear the genetically marked HxH, HxW, and WxW groups of fish (.32,000 per group), spawned in Task I.1.1, together in hatchery troughs or ponds at Clearwater Hatchery using standard hatchery rearing practices. Rear the fish in at least two raceways.

Task I.2.2. Compare the relative survival and size of experimental fish immediately before the standard release date for Clearwater Hatchery.

Task I.2.3. Mark the experimental fish at Clearwater Hatchery so that they can be identified when they return as adults. Mark a subset with PIT tags for evaluating downstream migration. Release the juveniles, at the standard time. Determine relative return rates as juveniles to PIT tag detectors at mainstem dams and as returning adults, and body sizes for these groups when the fish return as adults.

Task I.3.1. Develop length-fecundity and weight-fecundity relations for hatchery fish and for wild fish spawned for Objectives I.1 and I.2. Determine whether the size of eggs differs between hatchery fish and wild fish.

Task I.3.2. Capture adult fish returning to Crooked River. Release radio-tagged adults with the appropriate (homozygous) PEPA genotypes into Silver Creek or Crooked River above a weir to prevent them from leaving the stream before spawning. Estimate the number of eggs in hatchery females and in wild females released above the barriers. Using radio telemetry, ascertain whether any adults leave the study section before spawning, and whether there are differences in spawning location and habitat between hatchery and wild fish.

Task I.3.3. Sample the juveniles, offspring of the hatchery and wild adults released into the study sections, residing in each stream at the end of each growing season to determine relative reproductive success and size of the offspring of hatchery fish and wild fish. Trap the juveniles that migrate downstream, and test for differences in migration between groups.

Task I.4.1. At Dworshak NFH, mate adults heterozygous at the PEPA locus. Release some of the resulting fish in a study stream in the Palouse River drainage, and rear the remainder in the hatchery.

Task I.4.2. Take a sample of juvenile fish from the study stream at the end of each growing season, and determine the mean size and relative abundance of fish with the different genotypes.

Task I.4.3. Compare the relative survival and size of experimental fish with the different genotypes immediately before the standard release date at the hatchery.

Task I.4.4. Determine relative return rates and sizes of fish with the different genetic marks when they return to the hatchery as adults.

Task I.5.1. At Dworshak NFH, spawn adults on the appropriate dates, and incubate at the respective temperatures to achieve button-up simultaneously. Mark the otoliths of the juveniles thermally. Release approximately one-half of the resulting fish (from early and from late spawnings) as unfed fry in a study stream in the Palouse River drainage, and

rear the remainder in the hatchery.

Task I.5.2. Take a sample of juvenile fish from the study stream at the end of each growing season, and determine the mean size and relative abundance of fish from the two treatments (early, .7EC vs. late, .12EC).

Task I.5.3. Compare the relative survival and size of early and late experimental fish immediately before the standard release date at the hatchery.

Task I.5.4. Determine relative return rates and sizes of fish with the different genetic marks when they return to the hatchery as adults.

Task I.6.1. Take milt from at least 12 hatchery males. Divide the milt into two approximately equal portions; cryopreserve one portion and hold the other portion in plastic bags filled with oxygen and held on ice. Take the eggs from the same number of female hatchery fish. Divide the eggs from each female into two equal portions, and fertilize one portion with the cryopreserved milt from one male, and the other portion with Afresh \equiv milt from the same male. Use a different male with each female.

Task I.6.2. Split each treatment (fresh or cryopreserved milt) group for each mating, and incubate separately at WFRC until button-up. Divide each family and assign to rearing vessels so that each family is represented equally in each vessel. Two sets of tanks will contain fish from both treatments from all families, distinctively (marked by treatment) with mirror fin clips. One set of these mixed tanks will receive high ration levels (1.5 times the level recommended by the feed manufacturer). The other set will be fed at one-half of the recommended level. The remaining fish will be reared separately by treatment (with all families equally represented in each tank), and fed at the high level.

Task I.6.3. Monitor the growth and survival, by treatment, of the fish in the mixed tanks, and test for differences between treatments. During December-February, as the fish approach age-1, divide the fish from the unmixed tanks into replicate vessels, and evaluate (1) time to death and percent mortality for fish subjected to Vibrio anguillarum, blood cortisol levels of fish (2) held in a dip net out of the water for 60 seconds (acute handling stress) and (3) fish held at high density in buckets with approximately 5 cm of water for various periods, up to 48 hours, and blood sodium levels of fish held in sea water for 24 hr.

Task II.1.1. Capture hatchery and wild adults, combine their gametes to create genetically marked groups of HxH, H'xH', and WxW fish at the s-SOD-1 locus, and release these experimental groups of fish as eyed embryos or swim-up fry in the Little White Salmon River, and the Metolius River if available to us.

Task II.1.2. Sample juveniles residing in the study streams at the end of the growing season, and trap juveniles migrating downstream. Determine relative survival and size of the three groups of fish.

Task II.2.1. Rear genetically marked HxH, HxW, and WxW groups of fish, spawned in Tasks II.1.1 together in hatchery troughs or ponds at Warm Springs NFH using standard hatchery rearing practices. Rear the fish to the standard time of release.

Task II.2.2. Compare the relative survival and size of experimental fish shortly before the standard release date.

Task II.2.3. Mark the experimental fish (a clipped fin other than the adipose fin or some other external mark) so that they can be recognized when they return as adults. Release the juveniles when they reach the smolt stage of development. Determine relative return

rates and sizes of these fish when they return as adults.

Task II.3.1. At Warm Springs NFH, mate hatchery adults heterozygous at the s-SOD-1 locus. Release some of the resulting fish in a tributary of the Big White Salmon River, and rear the remainder in the hatchery.

Task II.3.2. Take a sample of juvenile fish from the study stream at the end of the growing season, and determine the mean size and relative abundance of fish with the different genotypes.

Task II.3.3. In the hatchery, compare the relative survival and size of experimental fish with the different genotypes shortly before the standard release date.

Task II.3.4. Evaluate the growth and survival in seawater for smolts from the various crosses. This work will be done at WFRCS Marrowstone Field Station, Washington.

Task II.3.5. Determine relative return rates and sizes of hatchery-reared fish with the different genetic marks when they return to the fisheries or the hatchery as adults.

Task II.4.1. Combine the gametes of adult fish from Carson NFH (H) and Warm Springs NFH (H') to create groups of HxH, HxH', H=xH, and H'xH' fish (female represented by the first symbol of each pair) at the s-SOD locus. One set of hybrid crosses (WSF) will use females from Warm Springs NFH and males from Carson NFH; the other set (CF) will use males from Warm Springs NFH and females from Carson NFH.

Task II.4.2 Apply distinctive thermal marks to the otoliths of the HxH, HxH', H=xH, and H'xH' groups of fish spawned in Task II.4.1, and rear them together hatchery ponds at Warm Springs NFH using standard hatchery rearing practices. Rear the fish to the standard time of release.

Task II.4.3. Compare the relative survival and size of experimental fish immediately before the standard release date.

Task II.4.4. Evaluate the growth and survival in seawater for smolts from the various crosses. This work will be done at WFRCS Marrowstone Field Station, Washington.

Additional details and justification of sample sizes are given in the original study proposal, and are not repeated here. We expect a statistical power of 25% with a 10% probability of type I error and a 20% chance of type II error.

Critical assumptions:

We assume that the genetic marks are selectively neutral, that differences observed between experimental groups result from genetic differences not maternal effects, and that incubation at different temperatures doesn't differentially effect subsequent growth or survival of experimental fish. Results from other studies suggest that the first two assumptions are valid; however, we have included ancillary tests in our study to evaluate each of these assumptions for our particular circumstances.

We assume that sufficient numbers of adults will return so that adults will be available for our study. Adequate numbers of returning adults must be available from wild populations and hatchery populations for us to initiate each complete replicate (year-class). These numbers may vary from year to year, and are determined, in consultation with us, by the Confederated Tribes of the Warm Springs Reservation (hatchery and wild spring chinook salmon), Idaho Department of Fish and Game and Perce Tribe (wild steelhead), and U.S.

Fish and Wildlife Service (hatchery steelhead and spring chinook salmon).

f. Facilities and equipment.

All offices, computers, laboratory and hatchery facilities, downstream migrant traps, and fish sampling equipment have proven adequate for the job, and should continue so for the duration of the project. The only "special" equipment yet to be purchased are a radio-telemetry receiver and about 80 radio tags for Objective I.3 (Reproductive success), Task I.3.2..

g. References.

Chilcote, M.W., S.A. Leider, and J.J. Loch. 1986. Differential reproductive success of hatchery and wild summer-run steelhead under natural conditions. *Transactions of the American Fisheries Society* 115(5): 726-735.

Hindar, K., N. Ryman, and F. Utter. 1991. Genetic effects of cultured fish on natural fish populations. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 945-957.

Independent Science Group. 1996. Return to the River. Northwest Power Planning Council, Portland, OR. Prepublication copy.

Leider, S.A., P.L. Hulett, J.J. Loch, and M.W. Chilcote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead trout through the returning adult stage. *Aquaculture* 88(3-4): 239-252

National Research Council. 1996. Upstream: salmon and society in the Pacific Northwest.

Nickelson, T.E., M.F. Solazzi, and S.L. Johnson. 1986. Use of hatchery coho salmon (*Oncorhynchus kisutch*) presmolts to rebuild wild populations in Oregon coastal streams. *Canadian Journal of Fisheries and Aquatic Sciences* 43(12): 2443-2449.

Reisenbichler, R.R., and J.D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. *Journal of the Fisheries Research Board of Canada* 34(1): 123-128.

Section 8. Relationships to other projects

Project 89-096, A genetic monitoring and evaluation program for supplemented populations of salmon and steelhead in the Snake River basin--Project 89-096 monitors the frequencies of (nearly) selectively neutral alleles (allozymes), in part, to estimate the reproductive success of hatchery fish used in supplementation programs. Study 90-052 handsomely complements 89-096 by providing information about the effect of such reproductive success on the fitness (or health), production, and productivity of the population of naturally spawning fish.

Project 89-098, Salmon supplementation studies in Idaho rivers--Project 89-098 evaluates the success of ongoing supplementation programs for chinook salmon. Because long-term effects, although potentially very serious, may not be discernable for eight or nine generations (30+ years; after Reisenbichler, in press), project 89-098 will be focussed on the short-term effects for the first two decades. Study 90-052 complements 89-098 by dealing with an additional species (steelhead), by evaluating genetic differences between hatchery and wild fish which allow extrapolation to the long-term consequences of supplementation, and by testing for the feasibility of modifying hatchery programs to reduce genetic problems associated with supplementation.

Project 90-055, Steelhead supplementation studies in Idaho rivers--Project 90-055 is to describe life history features of wild steelhead populations in Idaho. The weir that we designed and built under Project 90-052 for Fish Creek, Clearwater River system, is now being used for Project 90-055. The weir and the data that we collected with it during the first two years of this study are proving to be a cornerstone for Project 90-055. Idaho Fish and Game personnel consider 90-052 as the desirable, sister study to 89-098 and 90-055.

Kalama River study, funded by NMFS -- The Kalama studies and ours (90-052) complement each other by testing for differences in fitness between hatchery and wild fish, using hatchery fish that have been subjected to domestication selection for different numbers of generations, and from different Araces of steelhead (coastal, winter-run for the Kalama; inland, summer-run for us). It is necessary to pool data from these two studies and from previous work to develop a believable description for the rate of domestication from hatchery rearing. Some of the differences between the two studies are that our=s provides (1) a comparison of hatchery and wild fish both originating in the same (Clearwater) river system and of "single-stock" ancestry, (2) direct tests of domestication selection (i.e., comparisons in the hatchery as well as in streams), and (3) a comparison of hatchery and wild spring chinook salmon.

Other existing or planned studies similar to 89-098 (Supplementation programs in the Grande Ronde River system), funded by various agencies--Study 90-052 complements these studies in the same ways that it complements 89-098 or 90-055. Study 90-052 is the only one among these studies that elucidates long-term effects by specifically evaluating genetic differences between hatchery and wild fish, and excludes confounding with behavioral, physiological, or other direct effects of the hatchery programs.

Section 9. Key personnel

Reg Reisenbichler, fishery research biologist, contributes 0.5 FTE to this project. Reg designed the study, and oversees and participates in collection and analysis of data, budgeting, and report and manuscript preparation.

Steve Rubin, fishery research biologist, devotes 1.0 FTE to this project. Steve is

responsible for conducting the study. He assists in the overall planning, and is responsible for data collection, summary, and analysis, and report preparation. He is responsible for the staff of six or more persons and the planning and operation of day-to-day activities in the laboratory and the field.

Resume for Dr. Reginald R. Reisenbichler

Current Position: Fishery research biologist in population ecology at Western Fisheries Research Center, Biological Resources Division, USGS, 6505 NE 65th St., Seattle, WA, 98115.

Education:

B.S. (cum laude) in Zoology, minor in mathematics, Oregon State University. 1972.

M.S. in Fishery Biology, minor in statistics, Oregon State University. 1976.

Ph.D. in Fisheries Science (popn dynamics, statistics), University of Washington. 1986.

Recent positions:

1977-80 -- fishery biologist, U.S. Fish and Wildlife Service, Lander, Wyoming, and Red Bluff, California.

1980-present -- fishery research biologist, U.S. Fish and Wildlife Service, National Biological Service, or U.S. Geological Survey, Seattle, Washington.

1989-present -- Affiliate Assistant Professor, School of Fisheries, University of Washington.

Current responsibilities: Design and conduct research in the population ecology of anadromous salmonids and endangered species in the western United States.

Work experience in fisheries: Management and study of resident fish species in Wyoming and of anadromous Pacific salmonids in the Central Valley of California (3 years). Research in statistics and experimental design, and in population genetics, population dynamics, stream ecology, and life histories of anadromous Pacific salmonids from California to Alaska (20 years).

Partial list of publications:

Reisenbichler, R.R., and J.D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, Salmo gairdneri. Journal of the Fisheries Research Board of Canada 34: 123-128.

Reisenbichler, R.R., and N.A. Hartmann, Jr. 1980. Effect of number of marked fish and years of repetition on precision in studies of contribution to a fishery. Canadian Journal of Fisheries and Aquatic Sciences 37: 576-582.

Reisenbichler, R.R., J.D. McIntyre, M.F. Solazzi, and S.W. Landino. 1992. Genetic variation in steelhead of Oregon and northern California. Transactions of the American Fisheries Society 121: 158-169.

Reisenbichler, R.R., and G.S. Brown. 1995. Is Genetic Change From Hatchery Rearing of Anadromous Fish Really a Problem? American Fisheries Society Symposium 15:578-579.

Reisenbichler, R.R. 1997. Genetic factors contributing to declines of anadromous salmonids in the Pacific Northwest. Pages 223-244 in D.J. Stouder, P.A. Bisson, and R. J. Naiman [eds.] Pacific Salmon and Their Ecosystems: Status and Future Options. M.G. Duke [assoc. ed]. Chapman & Hall, Inc., New York.

Resume for Steve P. Rubin

Education: M.S. in Fishery Resources, University of Idaho, 1990; B.S. in Fisheries Science, Oregon State University.

Current employer (since June 1991): U.S. Geological Survey, Biological Resources Division, Western Fisheries Research Center, 6505 N.E. 65th St., Seattle WA 98115, 206-526-6282 ext. 324.

Title/grade: Research Fishery Biologist, GS-11.

Duties: Responsible for conducting a large, complicated study to quantify genetic differences between hatchery and wild fish from several populations of anadromous salmonids in Idaho, Oregon, and Washington. Plans, participates in, and oversees the conduct of associated laboratory experiments and field comparisons. Supervises permanent, temporary, and seasonal subordinates, and coordinates with personnel from other state, federal, and tribal agencies. Prepares reports and scientific papers on results of research projects.

Previous employment: Research Associate, Idaho Cooperative Fish and Wildlife Research Unit, 8/90-5/91; Graduate Assistant, Idaho Cooperative Fish and Wildlife Research Unit, 3/86-7/90; Experimental Biology Aide, Oregon Department of Fish and Wildlife, 10/85-2/86; Biological Technician, U.S. Forest Service, 7/85-9/85.

Expertise: Extensive knowledge of Columbia River summer steelhead and spring chinook salmon populations, life histories, and habitat use. Considerable experience making experimental matings for genetic studies including screening adults for genetic markers, planning crosses to match within-group genetic variation, stripping and mixing gametes with proper fertilization protocols, and cryopreserving milt for delayed fertilization. Comprehensive knowledge of field sampling protocols for salmonids in streams including designing and operating adult traps (picket weirs) and juvenile outmigrant traps, electrofishing, seining, snorkel surveys, measuring habitat, estimating population size and survival, and quantifying behavior and habitat use. Experience with a variety of marking and tagging methods including PIT, Floy, coded wire and jaw tags, thermally induced otolith marks, and genetic marks. Experience with aging techniques using scales and otoliths. Knowledge of statistical techniques and software (SAS, Systat).

Publications: Rubin, S. P., T. C. Bjornn, and B. Dennis. 1991. Habitat suitability curves for juvenile spring chinook salmon and steelhead developed using a habitat-oriented sampling approach. *Rivers* 2:12-29.

Co-author on annual reports (1992-present, in preparation) for the Performance/Stock Productivity Impacts of Hatchery Supplementation study, U.S. DOE, Bonneville Power Administration, Contract DE-AI79-91BP17760, Project No. 90-052.

Section 10. Information/technology transfer

Our findings will be distributed through final reports to Bonneville Power Administration and manuscripts published in peer-reviewed journals, and in oral presentations at workshops and conferences.